

The Antimicrobial Activity of Honey on Bacterial Isolates from Burns/Wound of Patients Attending General Hospital, Ankpa, Kogi, Nigeria

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Abstract

The antimicrobial activity of honey samples from Ankpa, Enjema and Ojoku districts of Ankpa Local Government Area of Kogi State against coagulase negative *Staphylococcus species*, *Pseudomonas aeruginosa*, coagulase positive *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli* and *Proteus species* from 200 burns/wound patients attending General Hospital, Ankpa were determined. The sensitivity of honey to the test organisms ranges from 16mm to 19mm in diameter. The minimum inhibitory concentration (MIC) of the honey samples on the bacterial isolates from Ankpa and Ojoku were 0.16 v/v and 0.32 v/v for honey from Enjema. The results of the study revealed that honey from the area under study has high antimicrobial activity hence could provide alternative antimicrobial agent to overcome the problem of increasing bacteria resistance to synthetic antimicrobial agents. It is therefore recommended that pure natural honey should be stocked in hospitals/clinics in order to encourage its application in the treatment of burns/wound infections.

Introduction

Over the years, the use of natural preparations from plants for the treatment of various ailments has been practiced by people of diverse cultures. Many of these natural preparations have been described as natural God – given foods for the good health of the body (Boom, 2004). He noted that treatment with chemicals (chemotherapy) seems to have been found to be inadequate and insensitive to many potential disease-causing micro-organisms. They developed resistance and this accounted for why naturopathic movements of the ancient time have blossomed from the 1990s. Many people are continually searching for alternative and more natural cures to alleviate the disease conditions and ailment of man. These natural remedies employed are biological in origin and are often more in form of food that could easily enter the body system and structure. They therefore play active parts in the physiological functions and balance as nature intends. Prominent among these natural remedies is honey.

According to World Health Organization estimates, about 60 percent of people living in developing countries rely solely on plants and plant products for their primary health care and the use of antibiotics constitute a sizable fraction of medicines consumed. The resistance to antimicrobial agents has become a serious issue in many areas in the world especially in developing countries. (Chauhan *et al.*, 2010). This honey has been identified and exploited as one of the popular natural antimicrobial substances. Honey (*Apis mellifera*) is the natural sweet substance produced by bees from nectar or the secretions of plants. It can be kept for a long period of time without becoming spoiled (Gbanem, 2011). It is used for the management of wounds and injuries. According to Subrahmanyam *et al.*, 2001, the medicinal properties of honey have been known since ancient times and have been described as the nectar of life. Its activity is mainly due to low pH, osmolality and hydrogen peroxide accumulation (Alnaimat *et al.*, 2012). The treatment with honey is called apitherapy, it has been recommended for various ailments which include replenishing energy, enhancing physical stamina and improving immune systems. Honey is considered to have calming effect on the mind and promotes sleep. It also helps digestion and is effective in the treatment of cardiovascular disease and respiratory complaints. Honey as an ancient remedy for the treatment of infected wounds has now been rediscovered by the medical profession; particularly where conventional modern therapeutic agents are failing. The current prevalence of microbial resistant to antibiotic has led to a re-evaluation of the therapeutic use of ancient remedies like honey.

Many people in the developing countries like Nigeria depend on local medicinal plants as remedy for their diseases and illnesses probably either because of the absence of modernized functional health care facilities or due to traditional and ancestral beliefs. The search for compounds with antimicrobial activity has gained increasing attention in recent time due to growing worldwide concern about the alarming increase in the rate of infection by antibiotic resistant microorganisms. These resistance of microorganisms to antibiotics and chemotherapeutic agents is as result of the use of under dosage, poverty in the population and the inability of people to purchase the complete dosage which result in the usage of sub- lethal dosage and development of resistance strains in bacteria.

The study was limited to the determination of antimicrobial assay on bacterial isolates from burns/wound of two hundred (200) patients attending the General hospital, Ankpa, Kogi State. The study took a period of three (3) months. The general aim of this study is to determine a novel therapeutic agent for the

treatment of infectious burns/wound among patients attending General Hospitals, Ankpa Kogi State. The specific objectives are as follows:

1. To characterize bacterial isolates from burns/wound of patients attending General Hospital, Ankpa in Kogi State.
2. To determine the antimicrobial activity and minimum inhibitory concentration (MIC) of honey on the bacterial isolated from the burns/wound of patients attending General Hospital, Ankpa Kogi State.

Due to the high cost of production and importation of antibiotics, the findings of this study would be important as it would provide alternative antimicrobial agent to overcome the problems of increasing bacterial resistance to synthetic antimicrobial agents and the high cost of production and importation of these drugs. There is therefore, the need to look inward for a natural approach and less expensive way of handling burns/wound infections.

Materials and Methods

Sample Collection

Pure and undiluted honey was purchased from bee producer in Ankpa, Enjema and Ojoku districts of Ankpa Local Government Area of Kogi State.

Preparation of honey Sample

The honey was first filtered with sterile metal sieve and placed in a sterile universal container in water bath at 60°C for 30mins. The honey solution was handled aseptically and protected from bright light to prevent photo-degradation of the glucose oxidase enzyme.

The isolation of test Organisms (Bacterial Isolates)

The isolation of test bacteria was obtained from swabs of 200 burns/wound patients that attended General hospital, Ankpa in Kogi State. The study took a period of three months from April 2011 to July, 2011. The burns/wound swabs were inoculated onto nutrient agar (Oxoid), plates and incubated at 37°C for 24 hour. After the end of incubation period, colonies were grouped and selected for Gram's staining and biochemical characterization.

Purification of isolates

The streak plate technique described by Ogbo (2005) was adopted for the purification of the isolates. A wire loop was flamed in Bunsen burner and a loop full of bacterial colony was picked and inoculated on the nutrient agar close to the edge of the Petri dish. The loop was sterilized and was used to streak from the inoculated spot to make streak 1. The loop flamed again and cooled, the streaking continued from the end of streak 1 to 2. This procedure was repeated until streak 5 was made. The Petri dish lid was finally replaced and the plate was incubated at 37°C for 24 hours.

The bacterial discrete colonies were isolated and sub-cultured onto nutrient agar (Oxoid), blood agar and mannitol salt agar plate and incubated at 37°C for 24 hours for isolation and purification of the test bacteria.

Antimicrobial activity of honey samples on bacterial isolates from burns/wound

The antimicrobial activity of honey was assayed using Agar well diffusion technique (Cheesbrough, 2004). A representative colony of each isolated bacteria was used for the antimicrobial assay.

A 0.1ml volume of the species was aseptically introduced into the nutrient agar (Oxoid) plates (one bacterial Isolate per two plates). The cultures were uniformly distributed all over the agar plate with the aid of a sterile glass spreader. The inoculated plates were allowed to dry and sterile cork borer were used to bore holes on each agar plate. The holes were sufficiently spaced and labeled accordingly with numbers.

A 0.1ml volume of the honey samples were introduced into the holes using a sterile Pasteur pipette. The inoculated plates were allowed to stand for one hour to ensure proper diffusion of the honey into the medium and incubated at 37°C for 24 hours. After incubation, the plates were observed for inhibition zones around the holes.

Determination of minimum inhibitory concentration (MIC) of honey samples

The minimum inhibitory concentration of honey samples was determined by a slight modification of the agar well dilution method as described by Omoregbe *et al.*, (1996) and Ogbo (2005). Double fold dilutions of the different samples of honey were made using a set of ten test tubes and sterile distilled water as diluent to determine the different honey concentrations in v/v of 5.0, 2.5, 1.25, 0.65, 0.32, 0.16, 0.08, 0.04, 0.02 and 0.01v/v. The varying honey concentrations between 5.0 to 0.01v/v were used to determine the MIC of honey on the bacterial isolates.

The MIC was determined on plates of nutrient agar (Oxoid) already inoculated with the different bacterial isolates. After drying, holes (each 10mm deep and 8mm in diameter) were bored on each agar plate

using a sterile cork borer. The holes were labeled accordingly with numbers. The holes were sufficiently spaced to avoid the zone of inhibition from over-lapping.

A 0.1ml of each of the honey samples were introduced into the holes accordingly using sterile Pasteur pipettes. The inoculated plates were allowed to stand for one hour to ensure proper diffusion of the honey into the medium and incubated at 37°C for 24 hours. After incubation, the plates were observed for inhibition zones around the holes. The plates were then examined and the diameter of the zone of inhibition was measured. The experiment was repeated three times for each bacterial isolate. The lowest concentration of the different sample that produced no zone of inhibition of growth of the test organism was taken as the minimum inhibitory concentration.

Results

Antimicrobial activity of honey on bacterial isolates from burns/wound samples

The results of the antimicrobial activity of honey samples from Ankpa, Ojoku and Enjema on bacterial isolates are presented on Table2 below:

The bacterial isolates were all sensitive to honey samples from Ankpa, Ojoku and Enjema districts in Ankpa Local Government Area of Kogi State (Table2). The degree of sensitivity of bacterial isolates were almost equal, ranging from 16 to 19mm diameter. Coagulase positive *Staphylococcus aureus* had the highest zone of inhibition of 19mm followed by coagulase negative *Staphylococcus species*, *Klebsiella Pneumoniae*, *pseudomonas aeruginosa* and *Proteus species* with 17mm while *Escherichia coli* had the lowest inhibition zone diameter of 16mm.

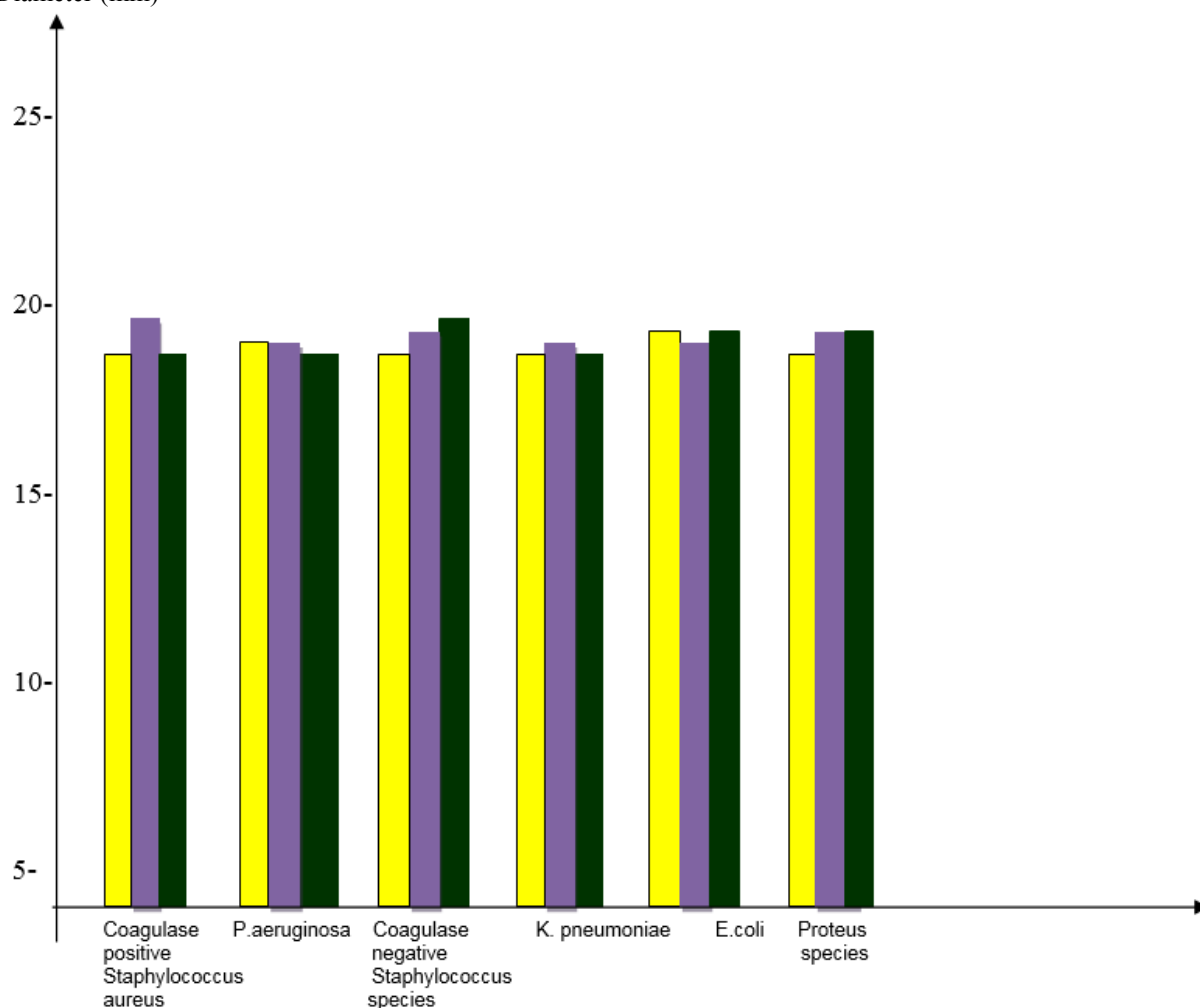
Table 1: Antimicrobial activity of honey samples against the bacterial isolates

Test Organism	Zone of inhibition diameter in millimetre (mm)		
	Honey A	Honey B	Honey C
<i>Coagulase-negative Staphylococcus aureus</i>	++	++	++
<i>Pseudomonas aeruginosa</i>	++	++	+
<i>Coagulase-positive Staphylococcus aureus</i>	++	++	+
<i>Klebsiella pneumoniae</i>	++	++	++
<i>Escherichia coli</i>	++	++	+
<i>Proteus species</i>	++	++	++

Source; July,2013

+++ :20mm and above ZID, ++:10 to 19mm ZID, +:1 to 9 mm ZID, 0: no inhibition, ZID: Zone of inhibition diameter in millimeter, honey A: Ankpa sample (N=3), honey B: Ojoku sample (N=3), and honey C: Enjema sample. (N=3).

Zone of inhibition
Diameter (mm)



Source; July, 2013

Figure 1: Comparison of the Relative Antimicrobial Activity of the Honey Samples

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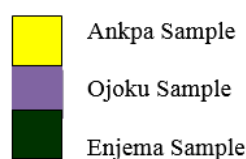


Figure 1 showed the comparison of the relative antimicrobial activity of the bacterial isolates to honey samples. The zone of inhibition diameter for coagulase positive *Staphylococcus aureus*, *Klebsiella Pneumoniae* and *Escherichia coli* with the three honey samples were markedly high. The same pattern was also observed with the Ojoku sample, for coagulase negative *Staphylococcus species*, Ankpa and Ojoku samples for *Pseudomonas aeruginosa*, and Ojoku and Enjema samples for *Proteus species*. However, the zone of inhibition in diameter for coagulase negative *Staphylococcus species*, *Pseudomonas aeruginosa* and *Proteus species* were lower with honey samples from Ankpa, and almost at the same level with honey sample from Enjema for coagulase negative *Staphylococcus species*. The degree of inhibition zone for standard tannin was observed to be 19mm for Coagulase positive *Staphylococcus aureus* while the other isolates had almost equal inhibition zone, ranging from 16-17mm.

Minimum inhibitory concentration (MIC) of Ankpa honey sample

The result of the minimum inhibitory concentration (MIC) of honey sample from Ankpa, Ojoku and Enjema on the bacteria isolates are presented in Table 3:

The minimum inhibitory concentration of Ankpa honey sample was 0.16v/v for all the bacteria isolates;

Proteus species, *Pseudomonas aeruginosa*, coagulase positive *Staphylococcus aureus*, *Escherichia coli*, coagulase-negative *Staphylococcus species*, and *Klebsiella pneumoniae*.

Table 2: Minimum inhibitory concentration (MIC) of Ankpa Honey sample in v/v

Test Organisms	MIC of Ankpa honey Sample in v/v and the degree of ZID									
	5.0	2.5	1.25	0.65	0.32	0.16	0.08	0.04	0.02	0.01
Coagulase negative <i>Staphylococcus aureus</i>	++	++	++	+	+	0	0	0	0	0
<i>Pseudomonas aeruginosa</i>	++	++	++	+	+	0	0	0	0	0
Coagulase-positive <i>Staphylococcus aureus</i>	+++	++	+	+	+	0	0	0	0	0
<i>Klebsiella pneumoniae</i>	++	++	+	+	+	0	0	0	0	0
<i>Escherichia coli</i>	++	++	++	+	+	0	0	0	0	0
<i>Proteus sp</i>	++	+	+	+	+	0	0	0	0	0

Source; July, 2013

++++: 20mm and above ZID, ++: 10-19mm ZID, +: 1 – 9mm ZID,

0: no inhibition, and ZID: zone of inhibition diameter (millimeter).

The MIC of honey sample from Ojoku was 0.16 v/v for all the isolates (*Proteus* species, coagulase positive *Staphylococcus aureus*, *Klebsiella pneumoniae*, coagulase-negative *Staphylococcus species*, *Pseudomonas aeruginosa* and *Escherichia coli*).

Table 3: Minimum inhibitory concentration (MIC) of Ojoku honey sample in v/v

Test Organisms	MIC of Ojoku honey Sample in v/v and the degree of ZID									
	5.0	2.5	1.25	0.65	0.32	0.16	0.08	0.04	0.02	0.01
Coagulase negative <i>Staphylococcus species</i>	++	++	+	+	+	0	0	0	0	0
<i>Pseudomonas aeruginosa</i>	++	++	+	+	+	0	0	0	0	0
Coagulase-positive <i>Staphylococcus aureus</i>	++	+	+	+	+	0	0	0	0	0
<i>Klebsiella pneumoniae</i>	++	+	+	+	+	0	0	0	0	0
<i>Escherichia coli</i>	++	+	+	+	+	0	0	0	0	0
<i>Proteus sp</i>	++	+	+	+	+	0	0	0	0	0

Source; July, 2013

+++ : 20mm and above ZID, ++: 10-19mm ZID, +: 1 – 9mm ZID,

0: no inhibition, and ZID: zone of inhibition diameter (millimeter.)

For the sample from Enjema, the MIC values was observed at the concentration of 0.16 v/v for all the bacterial isolates, (coagulase negative *Staphylococcus species*, coagulase positive *Staphylococcus aureus*, *Proteus species*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Escherichia coli*).

Table 4: Minimum inhibitory concentration (MIC) of Enjema honey samples in v/v

Test Organisms	MIC of honey Sample from Enjema in v/v and the degree of ZID									
	5.0	2.5	1.25	0.65	0.32	0.16	0.08	0.04	0.02	0.01
Coagulase negative <i>Staphylococcus species</i>	++	+	+	+	+	0	0	0	0	0
<i>Pseudomonas aeruginosa</i>	+	+	+	+	+	0	0	0	0	0
Coagulase-positive <i>Staphylococcus aureus</i>	++	++	+	+	+	0	0	0	0	0
<i>Klebsiella pneumoniae</i>	++	+	+	+	+	0	0	0	0	0
<i>Escherichia coli</i>	++	++	+	+	+	0	0	0	0	0
<i>Proteus sp</i>	+	+	+	+	+	0	0	0	0	0

Source; July, 2013

++++: 20mm and above ZID, ++: 10-19mm ZID, +: 1 – 9mm ZID,

0: no inhibition, and ZID: zone of inhibition diameter (millimeter).

The MIC of the standard tannin was 0.65v/v for *Proteus species* while coagulase negative *Staphylococcus species*, *Pseudomonas aeruginosa*, coagulase positive *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Escherichia coli* were 0.32 v/v.

Discussion

Six bacterial species were used in the course of this study. These were coagulase positive *Staphylococcus aureus*, coagulase negative *Staphylococcus species*, *Pseudomonas aeruginosa*, *klebsiella pneumoniae*, *Escherichia coli*, and *Proteus species*. The result of the antimicrobial effect of honey on the bacterial isolates using 100% concentration of honey samples from Ankpa and its environs inhibited the growth of all the isolates. The

inhibitory properties of the honey samples were established and these antimicrobial activities on the isolates are due to the presence of a number of properties inherent in honey which might contribute to its ability to fight infection and promote healing. Its high sugar content allows it to draw infection and fluid from wounds by a process called osmosis. Honey prevents bacterial growth through its low acidic pH and through the work of an enzyme (glucose oxidase) that produces small amount of hydrogen peroxide, responsible for antimicrobial activity. Honey may contain components from the specific plant used by the bees in their production, and it is speculated that some of these components might further add to the antibacterial effects of certain honey. These compounds include tannins, saponins, flavonoids, alkaloids, phenolic acid, and low acidic pH. The minimum inhibitory concentration of the honey samples from Ankpa and its environs shows MIC of 0.16 v/v.

Conclusions

Honey in Ankpa Local Government Area of Kogi State possesses antimicrobial activity. It is a potential source of alternative antimicrobial agent with a broad spectrum activity. The results of the study also support the traditional application of honey.

The antimicrobial activity of honey even at lower strength (minimum inhibitory concentration) of 0.16v/v, justified their efficacy in the treatment of burns/wound especially those associated with *Staphylococcus species*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Escherichia coli* and *Proteus species*.

Recommendations

It is therefore recommended that further work should be encouraged for the extraction of the crude components of honey and their antibiogram. Hospitals and clinics should be stocked with pure and undiluted natural honey so as to enhance its application in the treatment of burns/wounds. If these are done, it will encourage Nigerians in the use/application of honey as an excellent alternative antimicrobial agent for the treatment of burns/wound. This will reawaken and uplift the old practice of using honey to treat burns/wound at low cost.

References

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